

1 **Patent claims**

2 1. Process for the cultivation and stimulation of three-
3 dimensional, vital and mechanically-resistant cell
4 transplants in a GMP-conform bioreactor with the process
5 steps

6
7 a) the explant cells (12) taken from the organism and
8 prepared for bioreactor cultivation using methods which are
9 known and the carrier matrix (13) comprising commercially-
10 available biocompatible, absorbable or autologous or
11 homologous materials form a cell matrix suspension after
12 being mixed

13
14 b) placed in a seeding piston (25) which is foiled if
15 necessary and which has a cross-section which is adapted to
16 the later transplant, it being hardened out or polymerized
17 here,

18
19 c) if necessary, has minimum pressure applied to it by
20 means of an exactly fitting, inert stamp (26) which is
21 structured or foiled, if necessary,

22
23 d) the seeding piston with the transplant (11) is inserted
24 in the chamber space of the bioreactor carcass,

25
26 e) where the transplant (11) from the seeding piston (25)
27 is placed medially on the floor of the bioreactor and the
28 bioreactor (1) is closed after the seeding piston has been
29 removed,

30
31 f) where the transplant is subjected to further
32 cultivation by feeding a perfusion flow into it and

33
34 g) the replacement tissue material is removed for further
35 use after completion of the cultivation,

36

1 characterized in that the transplant is subjected to load in
2 the cultivation phase on the surface opposite the floor of
3 the bioreactor.

4
5 2. Process according to Claim 1, characterized in that the
6 transplant is loaded by a stamp which applies pressure.

7
8 3. Process according to claims 1 und 2, characterized in
9 that both the blending in the bioreactor chamber t due to
10 the perfusion flow and the stamp which applies pressure to
11 the transplant can be controlled with regard to time and
12 quantity or density in relation to the cultivation
13 conditions.

14
15 4. Process according to claims 1 - 3, characterized in
16 that the transplant has conditioned cultivating medium flow
17 through it at intervals and is subjected to loading in
18 cycles by the stamp which applies pressure.

19
20 5. Process according to Claims 1 - 4, characterized in
21 that the pressure load of the transplant takes place during
22 the perfusion.

23
24 6. Process according to Claims 1 - 5, characterized in
25 that the transplant is stimulated by using static,
26 preferably in-vivo-similar pressure loads or construct
27 deformations or they are continuously loaded by intermittent
28 or dynamic pressure forces.

29
30 7. Process according to Claims 1 - 6, characterized in
31 that the mechanical load is applied with a frequency
32 exceeding 0.1 Hz.

33
34 8. Process according to claims 1 - 7 characterized in that
35 the mechanical pressure stimulation is in the form of a
36 symmetrical or asymmetrical half cosine or sine wave.

1
2 9. Process according to claims 1-8 characterized in that
3 the pressure stamp which is from a magnetic material is
4 moved longitudinal to the surface of the transplant in the
5 bioreactor by an (electro-) magnetic field which is
6 generated outside the bioreactor.

7
8 10. Process according to claims 1 - 9, characterized in
9 that the magnetic field is generated by at least one
10 permanent magnet.

11
12 11. Process according to claims 1-10 characterized in that
13 at least two permanent magnets with alternating polarity are
14 positioned on a mobile holder above the bioreactor and is
15 driven by a servomotor in a cyclic manner, they thereby
16 changing their position resulting in the pressure stamp
17 applies pressure to the transplant alternately.

18
19 12. Process according to claims 1-8 characterized in that
20 the coil of an electromagnet changes alters its current
21 direction and voltage and therefore the field direction and
22 magnetic flow density with high frequency via the
23 bioreactor, resulting in the pressure stamp applying
24 pressure to the transplant alternately.

25
26 13. Bioreactor for the cultivation and stimulation of
27 three-dimensional, vital and mechanically resistant cell
28 transplants in an GMP-conform bioreactor,
29 characterized in that the bioreactor (1) of a basic carcass
30 with a reactor lock (21) is connected in a pressure proof
31 and sterile manner, this creating at least one reactor
32 chamber, a storage space for a transplant (11) and a mini
33 actuator (14) being implemented in this, the bioreactor (1)
34 being equipped with at least two hose coupling connections
35 (19) for the feeding and discharging of the medium in
36 addition to the gassing.

1
2 14. Device according to Claim 13, characterized in that the
3 cell culture constructs (11) can be directly or indirectly
4 cultivated and stimulated on the bioreactor floor of a
5 single-chamber bioreactor (1).

6
7 15. Device according to Claim 13 in that the cell culture
8 constructs (11) are directly or indirectly, at least
9 partially positioned on a floor of the upper reaction
10 chamber of a double-chamber bioreactor for cultivation and
11 stimulation, whilst this transplant (11) is in a second
12 reactor chamber.

13
14 16. Device according to Claims 14 or 15, characterized in
15 that the bioreactor (1) is equipped with a transplant insert
16 on the floor of the upper reactor chamber in which the cell
17 constructs (11) can be placed.

18
19 17. Device according to Claims 13 - 16, characterized in
20 that the container of the bioreactor (1) is a cylinder-
21 shaped corpus which is closed from above with a reactor lock
22 (21).

23
24 18. Device according to Claims 13 - 17, characterized in
25 that the reactor lock unit(s) (21) and the bioreactor (1)
26 are connected by one threaded joint and at least one conical
27 nipple (20) in such a way that the threaded joint is either
28 created between the reactor lock (21) and the container(1)
29 by a female thread in the container (1) and a male thread in
30 the reactor lock working together or the threaded joint is
31 created between the reactor lock (21) and the container (1)
32 in that a male thread in the container (1) and a female
33 thread in the reactor lock (21) work together.

34
35 19. Device according to Claims 13 - 18, characterized in
36 that the reactor lock (21) in the form of a cover is

1 equipped with biosensors (9) and/or measuring heads (10).

2

3 20. Device according to Claims 13 - 19, characterized in
4 that the cover (21) is equipped with a sample taking section
5 (10).

6

7 21. Device according to Claims 13 - 20, characterized in
8 that the basic carcass of the bioreactor (13) has at least
9 two each of a feed and discharge borehole for the provision
10 of hose coupling connections in the single-chamber
11 bioreactor version.

12

13 22. Device according to Claims 13 - 20, characterized in
14 that the basic carcass of the bioreactor (1) has at least
15 two boreholes for the provision of hose coupling connections
16 (19) in the double-chamber bioreactor version.

17

18 23. Device according to Claim 22, characterized in that at
19 least one hose coupling connection (19) is integrated in the
20 lower reaction chamber and at least one in the upper
21 reaction chamber.

22

23 24. Device according to Claims 21 - 23, characterized in
24 that the feed connections (19) and discharge connections
25 (19) which enter the bioreactor chamber are fitted with a 3-
26 way valve (6) or a 4-way valve (7) with a return function.

27

28 25. Device according to Claim 24, characterized in that at
29 least one of the discharge connections (19) is provided with
30 a sample taking section (10).

31

32 26. Device according to Claims 13 - 25, characterized in
33 that the bioreactor (1) has a reactor floor of a completely
34 or partially transparent material, preferably a transparent
35 glass plate (17) for the monitoring of the transplant
36 manufacture.

1

2 27. Device according to Claims 13 - 26, characterized in
3 that a foil; fleece or membrane (18) of an antistatic or
4 inert material is situated above the reactor floor of the
5 bioreactor (1) for the positioning of the transplant (11),
6 this material preferably being wide-meshed and light, fluid
7 and gas permeable.

8

9 28. Device according to one of the Claims 13 - 27,
10 characterized in that when in the version of a double-
11 chamber reactor, the upper reactor chamber of the bioreactor
12 (1) has an area which corresponds to the transplant area
13 whilst the dimensions of the lower chamber are under those
14 of the transplant (11) so that if a cell culture is placed
15 medially, this construct is mainly positioned underneath the
16 lower chamber and is partially on the reactor floor of the
17 upper chamber.

18

19 29. Device according to Claim 28, characterized in that the
20 space underneath the reactor chamber is filled out by a flat
21 plate (16) of a biologically inert, light-permeating, wide-
22 pored material, preferably of a porous sinter material so
23 that this plate (16) is flush with the floor of the upper
24 reactor chamber.

25

26 30. Device according to Claims 28 and 29, characterized in
27 that a foil, fleece or membrane (18) of an antistatic or
28 inert material is situated above the lower reactor chamber
29 which is filled out by the plate (16) on the reactor floor
30 of the upper reactor chamber of the double-chamber
31 bioreactor (1), this material being for the positioning of
32 the transplant (11), it preferably being wide-meshed and
33 permeable to light, fluid and gas.

34

35 31. Device according to Claims 28-30, characterized in that
36 the components which are underneath the transplant (11) in

1 the double-chamber bioreactor (1) such as the transparent
2 plate (17), the lower chamber with the inserted porous
3 material (16) and a wide-meshed membrane (18) have an
4 overall height which does not exceed the focal distance of
5 commercially available microscope and camera objectives.

6
7 32. Device according to Claim 13, characterized in that a
8 magnetic, preferably piston-like mini actuator (14) is
9 situated in the bioreactor (1) and can be moved through the
10 bioreactor (1) in a controlled manner by one or more
11 externally arranged control and steering magnets (15).

12
13 33. Device according to Claims 13-32, characterized in that
14 the mini actuator (14) in the single-chamber bio reactor (1)
15 is situated above the membrane (18) and the transplant (11)
16 in the bioreactor space.

17
18 34. Device according to Claims 13-32, characterized in that
19 the mini actuator (14) in the double-chamber bioreactor (1)
20 is situated in the upper reactor space above the porous
21 material (16), the membrane (18) and the transplant (11).

22
23 35. Device according to Claims 32-34, characterized in that
24 the magnetic mini actuator (14) which is to be implemented
25 comprises a magnetic nucleolus (22), preferably of a
26 permanent magnet which is encapsulated in a biologically
27 inert enveloping body (23), preferably plastic.

28
29 36. Device according to Claims 32-35, characterized in that
30 the magnetic nucleolus (22) is so oriented that the field
31 which is generated between the poles runs vertical to the
32 transplant (11) so that the magnetic north pole of the
33 complete mini actuator (14) is oriented in an upwards
34 direction.

35
36 37. Device according to Claims 32-36, characterized in that

1 the biocompatible enveloping body (23) which surrounds the
2 core magnets (22) has an external form which matches the
3 form of the reactor chamber of the bioreactor (1).

4
5 38. Device according to Claims 32-35, characterized in that
6 the complete height of the enveloping body (23) has been so
7 selected that an implementation of the mini actuator (14) in
8 the reactor space results in a vertically-oriented guiding
9 of the pressure stamp of the mini actuator (14) towards the
10 transplant (11).

11
12 39. Device according to Claims 32-38, characterized in that
13 the piston-shaped mini actuator (14) comprises more than one
14 enveloping body cylinder so that one of the enveloping body
15 cylinders, preferably the upper one contains the
16 encapsulated permanent magnet and an additional cylinder
17 serves the stamp impression (24), whereby the spatially
18 separated cylinders are joined by means of a bridge (34) or
19 a connection which has the same function.

20
21 40. Device according to Claims 32-39, characterized in that
22 the planar stamp surface (24) on the underside of the mini
23 actuator (14) formed by the enveloping body (23, runs
24 vertical to the guide direction in the bioreactor space (1).

25
26 41. Device according to Claims 32 and 40, characterized in
27 that organotypical negative forms such as a convex form are
28 embossed on the stamp surface (24) of the mini actuator
29 (14).

30
31 42. Device according to Claims 32 and 41, characterized in
32 that the planar or formed stamp surface (24) is embossed in
33 the form of a grid structure in order to increase the stamp
34 surface, this preferably having a small-meshed structure.

35
36 43. Device according to Claims 32 - 42, characterized in

1 that the enveloping body (24) of the mini actuator (14) is
2 to be so provided with drill holes and/or flow channels (33)
3 that a continued exact vertical guiding of the mini actuator
4 (14) is still guaranteed at 3 points.

5

6 44. Device according to Claims 32 - 44, characterized in
7 that the stamp surface (24) of the mini actuator (14) is
8 fitted with at least one nosepiece which slides into its
9 exactly fitting integrated guide rail in the bioreactor
10 carcass.

11

12 45. Device according to Claims 32 - 44, characterized in
13 that the control and steering magnet (15) situated outside
14 the bioreactor (1) brings about an oriented movement of the
15 implemented mini actuator (14) with the electro-) magnetic
16 field which it generates with the north pole of the
17 permanent magnet which is oriented upwards (22).

18

19 46. Device according to Claims 32 - 45 characterized in
20 that the control and steering magnet (15) is medially
21 situated in a vertical axis to the pressure stamp (14),
22 preferably above the pressure stamp (14) and moves upwards
23 and downwards in relation to the polarity of the mini
24 actuator (14), resulting in an alteration of the pressure
25 applied to the transplant (11).

26

27 47. Device according to Claim 32, characterized in that the
28 control and steering magnet (15) comprises two permanent
29 magnets (32) with different vertical magnetic pole
30 directions which are inserted in a rectangular shaped magnet
31 holder (31) and moved to their horizontal position above the
32 bioreactor in a cyclic manner by means of a servomotor (29)
33 and a guide rail (30).

34

35 48. Device according to Claim 32, characterized in that the
36 control and steering magnet (15) comprises a minimum of two

1 permanent magnets (32) with different vertical magnetic pole
2 directions, these being in a disk-shaped magnet holder (31)
3 and moved over the bioreactor (1) in a cyclic manner as a
4 result of the rotation drive of a servomotor (29).

5
6 49. Device according to Claim 32, characterized in that the
7 bioreactors which are firmly fixed in their horizontal
8 position approach the permanent magnets (32) via a vertical
9 movement of the magnet holder (31) by means of a step motor,
10 in order to increase the field effect and generate the
11 application of higher pressures on the transplant (11).

12
13 50. Device according to Claim 49, characterized in that at
14 least two bioreactors (11) are so arranged in a station that
15 their mini actuators (14) are driven by just one permanent
16 magnetic control system in a contactless manner.

17
18 51. Device according to Claim 32, characterized in that the
19 control and steering magnet (15) is in the form of an
20 electromagnet with at least one induction coil (35) with a
21 field which is infinitely variable using the known means.

22
23 52. Device according to Claims 32 and 51 characterized in
24 that the induction coil (35) is highly frequently triggered
25 by frequencies which can be altered in order to generate
26 high-dynamic magnetic field alterations and mini actuator
27 (14) movement on the transplant (11).

28
29 53. Device according to Claims 13 - 53 characterized in
30 that a seeding piston (25) which is preferably cylindrical
31 and has an inside diameter which corresponds to the outside
32 diameter of the transplants on a moving sliding plate (27)
33 for the purpose of injecting the cells (12) and the carrier
34 matrix (13).

35
36 54. Device according to Claims 13 - 53 characterized in

1 that the moving sliding plate (27) and the inside of the
2 seeding piston (25) is coated by an inert membrane, foil or
3 polymer fleece.

4

5 55. Device according to Claims 53 and 54 characterized in
6 that an exactly fitting stamp (26) with a planar stamp
7 surface or an organotypical negative form in the seeding
8 piston (25) is lightly applied to the cell suspension.

9

10 56. Device according to Claims 53 - 55 characterized in
11 that the outside diameter of the seeding piston (25) exactly
12 matches the inside diameter of the bioreactor (1).

13

14 57. Device according to Claims 13 - 56 characterized in
15 that at least 3 fixation walls (28) are integrated in the
16 reactor floor of the bioreactor (1), these having dimensions
17 which accommodate a transplant insert and do not impair the
18 pressure compression.

19

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23

1	List of Reference Drawings	
2		
3	1	Bioreactor
4	2	Medium reservoir
5	3	Supplement reservoir
6	4	Hose system
7	5	Circulation pump
8	6	3-way valve
9	7	4-way valve
10	8	Sample taking section
11	9	Biosensors Glu/Lac
12	10	Measuring heads pH, pCO ₂ , pO ₂
13	11	Transplant
14	12	Cells/tissue
15	13	Support matrix
16	14	Mini actuator
17	15	Control magnet
18	16	Porous sinter material
19	17	Transparent glass
20	18	Permeable membrane
21	19	Hose coupling/Luer connection
22	20	Pinch ring
23	21	Reactor lock
24	22	Permanent magnet
25	23	Enveloping body
26	24	Stamp surface
27	25	Seeding piston
28	26	Stamp
29	27	Sliding plate
30	28	Fixation wall
31	29	Servomotor
32	30	Guide rail
33	31	Magnet holder
34	32	Permanent magnet
35	33	Flow channels
36	34	Extension nosepiece
37	35	Coil

1 **Summary**

2

3 Process and Bioreactor for the Cultivation and Stimulation of
4 Three-dimensional, Vitally and Mechanically Resistant Cell-
5 Transplants

6

7 The task of the invention is the creation of a process and a
8 bioreactor for the manufacturing of three-dimensional, vital
9 and mechanically-resistant cell cultures, by which they can
10 be cultivated and stimulated within a short time of each
11 other or simultaneously. The bioreactor should permit GMP-
12 conform transplant cultivation under guaranteed sterile
13 conditions.

14

15 The bioreactor (1) comprises a basic carcass which is
16 connected to a reactor lock (21) so that it is pressure
17 proof and sterile, this creating at least one reactor
18 chamber, a storage space for a transplant (11) and a mini
19 actuator (14) being implemented in this. The bioreactor (1)
20 is also equipped with at least two hose coupling connections
21 (19) for the feeding and discharging of the medium in
22 addition to the gassing.

23

24 The invention enables GMP-conform cultivation of three-
25 dimensional, vital and mechanically-resistant cell cultures,
26 preferably cartilage-cell constructs which can hereby be
27 cultivated and stimulated in a locked mini-bioreactor
28 simultaneously, consecutively or within a time-controlled
29 process according to GMP guidelines. These so-called
30 transplants which are cultivated in this manner are then
31 available as replacement tissue material for the therapy of
32 connective and supporting tissue defects, direct joint
33 traumas, rheumatism and degenerative joint disease, for
34 example and can present an alternative to the conventional
35 (operative) therapy approaches, such as micro fracturing or
36 drill perforation in arthrosis of the knee joint, for

1 example.

2

3 Figure 3

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